

Figure 1A

2H7scFv-Ig cDNA and predicted amino acid sequence:

```
HindIII      NcoI      2H7 VL Leader Peptide→
~~~~~      ~~~~~
1  AAGCTTGCCG CC  M D F Q V Q I F S F L L I S A S
                        ATGGATT TCAAGTGCAG ATTTTCAGCT TCCTGCTAAT CAGTGCTTCA

                        2H7 VL→
61  V I I A R G Q I V L S Q S P A I L S A S
    GTCATAATTG CCAGAGGACA AATTGTTCTC TCCAGTCTC CAGCAATCCT GTCTGCATCT

    P G E K V T M T C R A S S S V S Y M H W
121 CCAGGGGAGA AGGTCACAAT GACTTGCAGG GCCAGCTCAA GTGTAAGTTA CATGCACTGG

                        BamHI
                        ~~~~~
181  Y Q Q K P G S S P K P W I Y A P S N L A
    TACCAGCAGA AGCCAGGATC CTCCCCCAA CCCTGGATTT ATGCCCCATC CAACCTGGCT

    S G V P A R F S G S G S G T S Y S L T I
241 TCTGGAGTCC CTGCTCGCTT CAGTGGCAGT GGGTCTGGGA CCTCTTACTC TCTCACAATC

    S R V E A E D A A T Y Y C Q Q W S F N P
301 AGCAGAGTGG AGGCTGAAGA TGCTGCCACT TATTACTGCC AGCAGTGGAG TTTTAAACCA

                        (Gly4Ser)3 Linker
361  P T F G A G T K L E L K G G G G S G G G
    CCCACGTTCG GTGCTGGGAC CAAGCTGGAG CTGAAAGGTG GCGGTGGCTC GGGCGGTGGT

                        2H7 VH→
421  G S G G G G S S Q A Y L Q Q S G A E L V
    GGATCTGGAG GAGGTGGGAG CTCTCAGGCT TATCTACAGC AGTCTGGGGC TGAGCTGGTC

    R P G A S V K M S C K A S G Y T F T S Y
481 AGGCCTGGGG CCTCAGTGAA CATGTCCTGC AAGGCTTCTG GCTACACATT TACCAATTAC

    N M H W V K Q T P R Q G L E W I G A I Y
541 AATATGCACT GGGTAAAGCA GACACCTAGA CAGGGCCTGG AATGGATTGG AGCTATTTAT

    P G N G D T S Y N Q K F K G K A T L T V
601 CCAGGAAATG GTCATACTTC CTACAATCAG AAGTTCAAGG GCAAGGCCAC ACTGACTGTA

    D K S S S T A Y M Q L S S L T S E D S A
661 GACAAATCCT CCAGCACAGC CTACATGCAG CTCAGCAGCC TGACATCTGA AGACTCTGCG

    V Y F C A R V V Y Y S N S Y W Y F D V W
721 GTCTATTTCT GTGCAAGAGT GGTGTACTAT AGTAACTCTT ACTGGTACTT CGATGTCTGG
```

Figure 1 **B**

BclI
~~~~~human IgG1 Fc domain →

781 G T G T T V T V S D Q E P K S C D K T H  
GGCACAGGGA CCACGGTCAC CGTCTCTGAT CAGGAGCCCA AATCTTGTGA CAAAACCTCAC

841 T C P P C P A P E L L G G P S V F L F P  
ACATGCCCAC CGTGCCACAGC ACCTGAACTC CTGGGGGGAC CGTCAGTCTT CCTCTTCCCC

901 P K P K D T L M I S R T P E V T C V V V  
CCAAAACCCA AGGACACCCT CATGATCTCC CGGACCCTG AGGTCACATG CGTGGTGGTG

961 D V S H E D P E V K F N W Y V D G V E V  
GACGTGAGCC ACGAAGACCC TGAGGTCAAG TTCAACTGGT ACGTGGACGG CGTGGAGGTG

1021 H N A K T K P R E E Q Y N S T Y R V V S  
CATAATGCCA AGACAAAGCC GCGGGAGGAG CAGTACAACA GCACGTACCG TGTGGTCAGC

1081 V L T V L H Q D W L N G K E Y K C K V S  
GTCTCTACCG TCCTGCACCA GGACTGGCTG AATGGCAAGG AGTACAAGTG CAAGGTCTCC

1141 N K A L P A P I E K T I S K A K G Q P R  
AACAAAGCCC TCCCAGCCCC CATCGAGAAA ACAATCTCCA AAGCCAAAGG GCAGCCCCGA

1201 E P Q V Y T L P P S R D E L T K N Q V S  
GAACCACAGG TGTACACCCT GCCCCATCC CGGGATGAGC TGACCAAGAA CCAGGTCAGC

1261 L T C L V K G F Y P S D I A V E W E S N  
CTGACCTGCC TGGTCAAAGG CTTCTATCCC AGCGACATCG CCGTGGAGTG GGAGAGCAAT

1321 G Q P E N N Y K T T P P V L D S D G S F  
GGGCAGCCGG AGAACAATA CAAGACCACG CCTCCCGTGC TGGACTCCGA CGGCTCCTTC

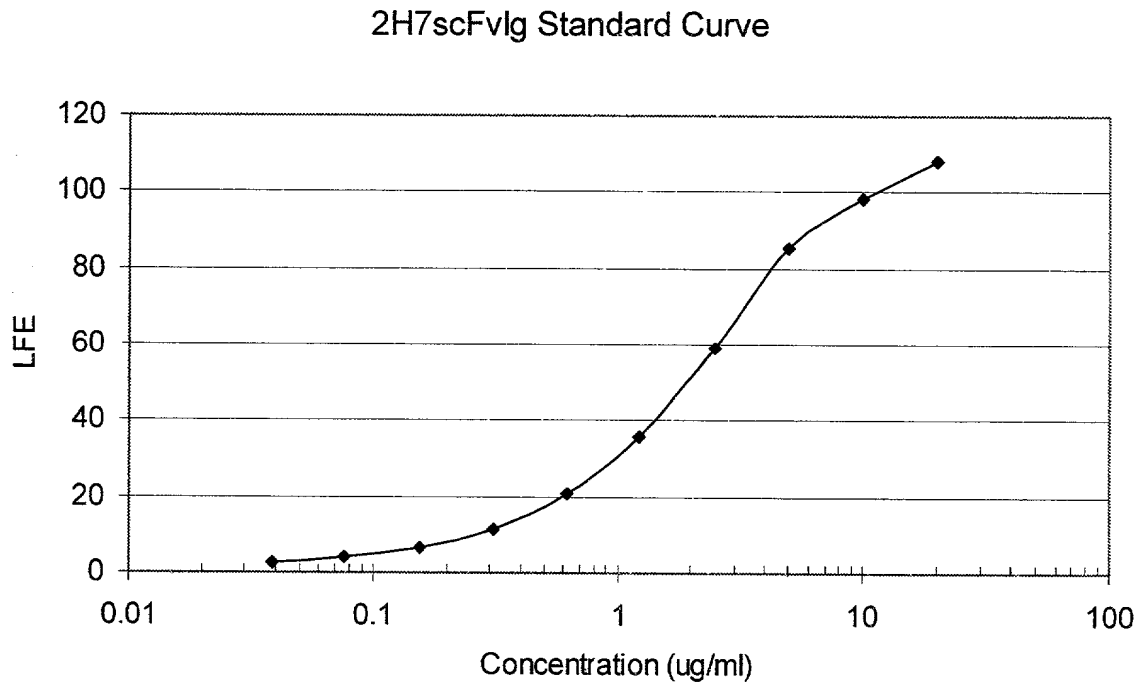
1381 F L Y S K L T V D K S R W Q Q G N V F S  
TTCCTCTACA GCAAGCTCAC CGTGGACAAG AGCAGGTGGC AGCAGGGGAA CGTCTTCTCA

1441 C S V M H E A L H N H Y T Q K S L S L S  
TGCTCCGTGA TGCATGAGGC TCTGCACAAC CACTACACGC AGAAGAGCCT CTCCCTGTCT

XbaI  
~~~~~

1501 P G K * S R
CCGGGTAAAT GATCTAGA

Figure 2.



Clone	LFE @ 1:50	Estimated Concentration (μ g/ml)
D2	26.1	56
IIIC6	25.7	55
IVA3	28.6	61
Spent bulk	29.6	64

Figure 3.

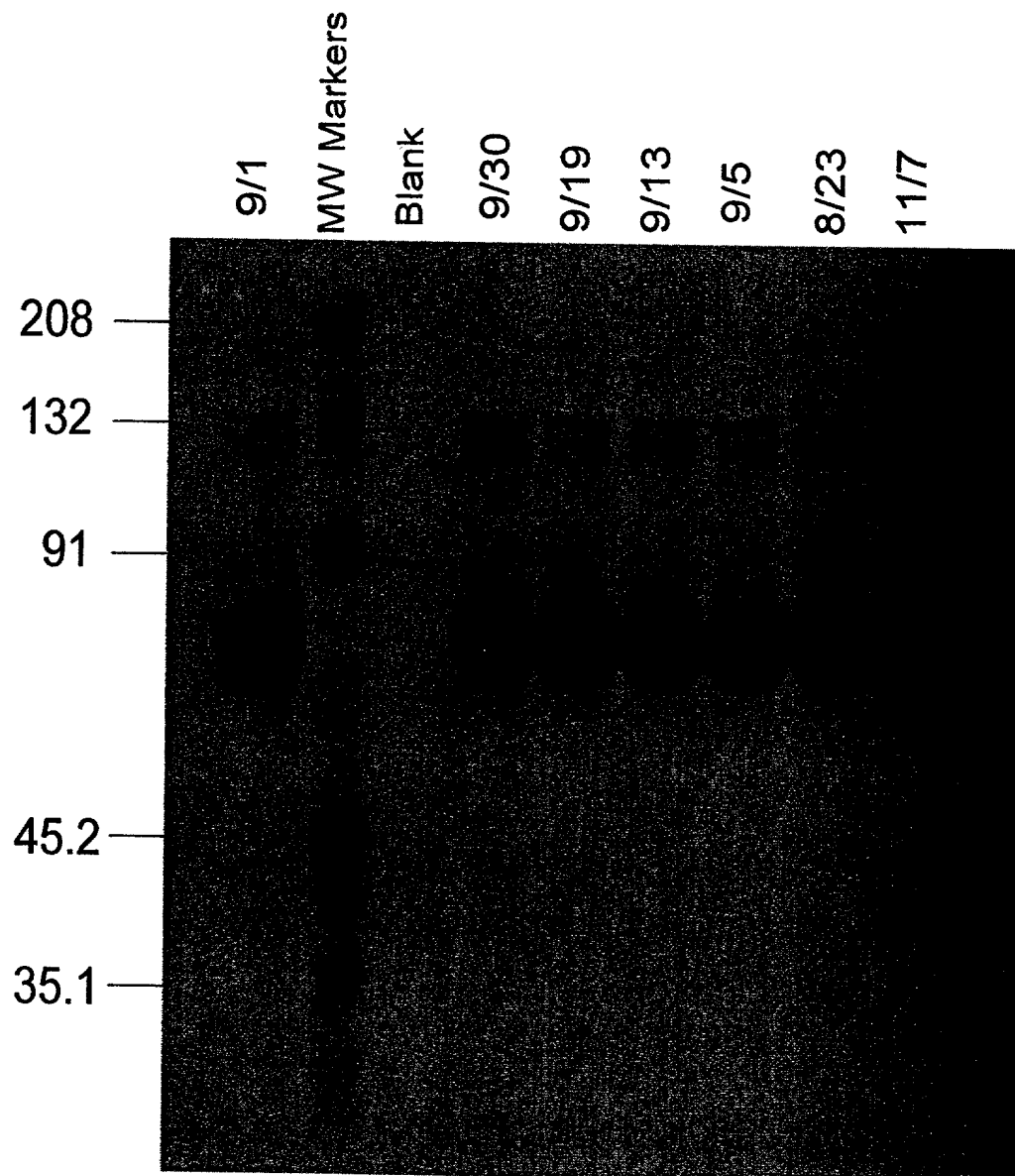


Figure 4A.

Complement Mediated B Cell Killing After Binding of CD20-targeted 2H7 Derivatives:

2H7scFv-Ig Concentration	RAMOS	BJAB
20 µg/ml + complement	0.16	0.07
5 µg/ml + complement	0.2	N.D.
1.25 µg/ml + complement	0.32	0.1
Complement alone	0.98	0.94

*Viability was determined by trypan blue exclusion and is tabulated as the fraction of viable cells out of the total number of cells counted.

**N.D. (not determined).

Figure 4B.

Antibody-dependent cellular cytotoxicity (ADCC) mediated by 2H7scFv-Ig:

ADCC Activity of 2H7scFv-Ig on Ramos Cells

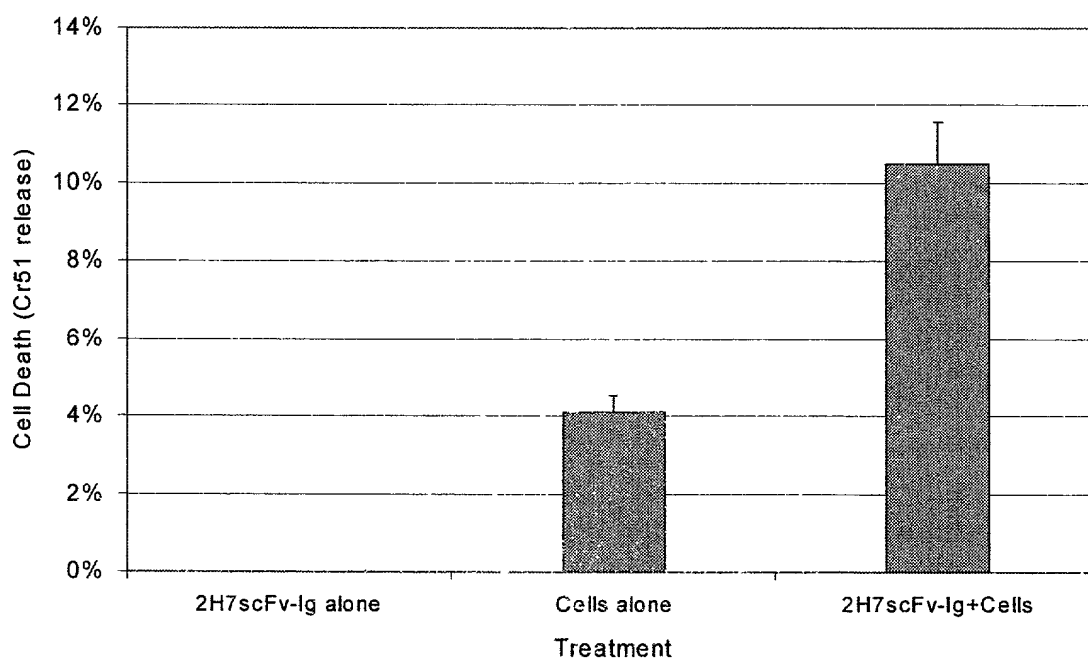


Figure 5.

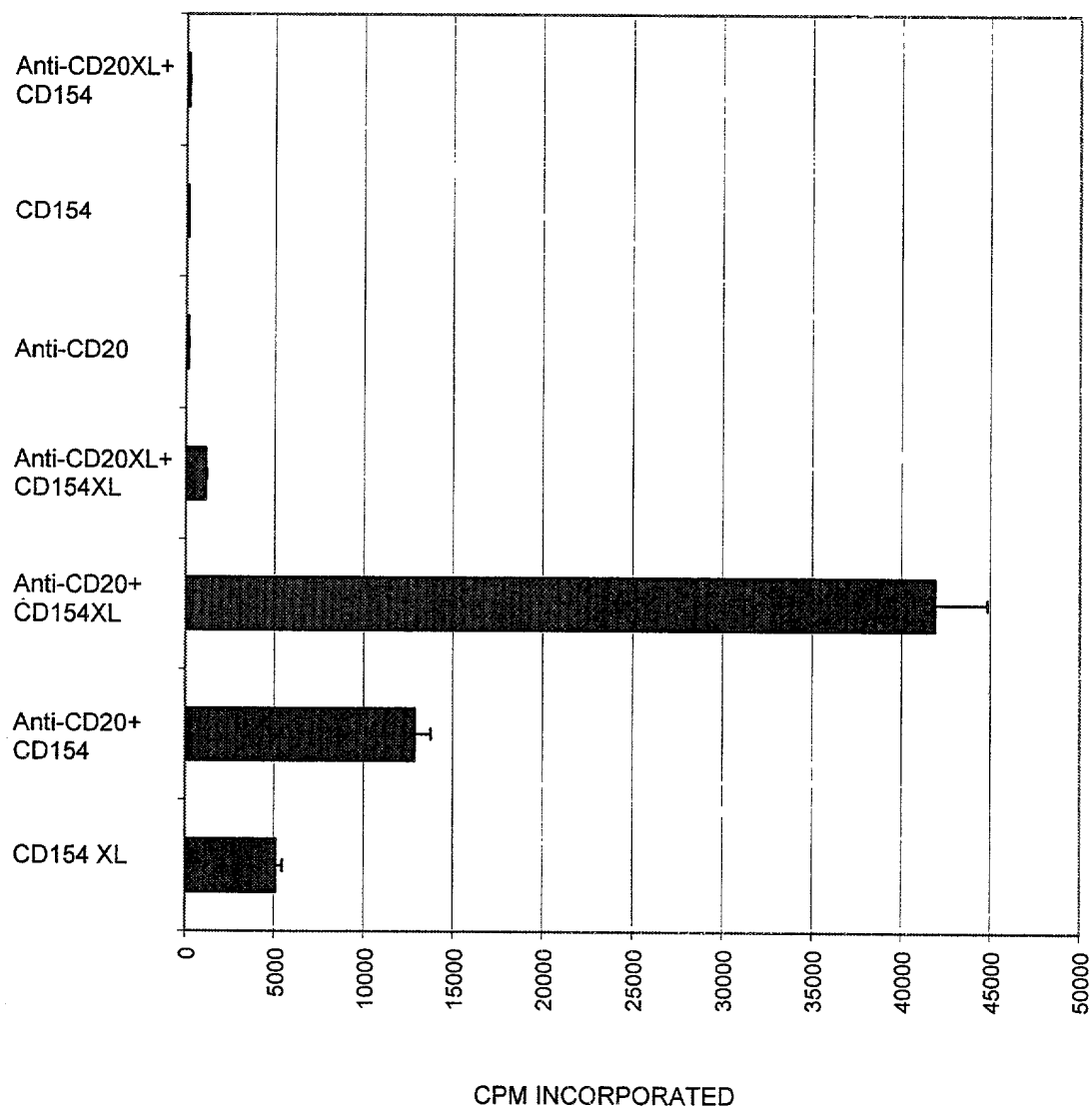


Figure 6A and B.

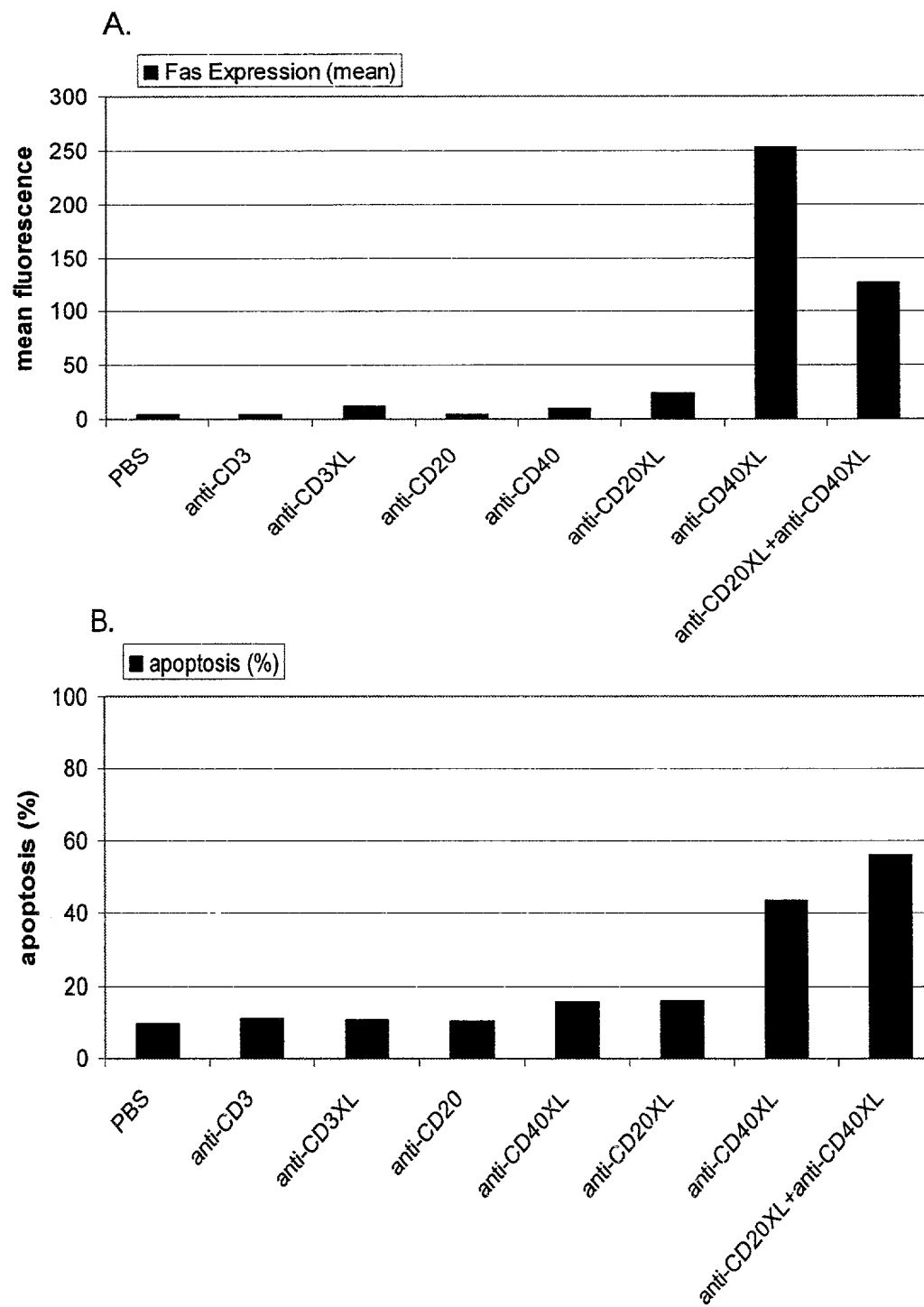


Figure 7A.

2H7-CD154 L2 cDNA and predicted amino acid sequence:

```
HindIII      NcoI   2H7 VL Leader Peptide →
~~~~~      ~~~~~
1  AAGCTTGCCG CC   M D F Q V Q I F S F L L I S A S
                        ATGGATT TCAAGTGCAG ATTTTCAGCT TCCTGCTAAT CAGTGCTTCA

                        2H7 VL →
61  V I I A R G Q I V L S Q S P A I L S A S
    GTCATAATTG CCAGAGGACA AATTGTTCTC TCCAGTCTC CAGCAATCCT GTCTGCATCT

    P G E K V T M T C R A S S S V S Y M H W
121 CCAGGGGAGA AGGTCACAAT GACTTGCAGG GCCAGCTCAA GTGTAAGTTA CATGCACTGG

                        BamHI
                        ~~~~~
181  Y Q Q K P G S S P K P W I Y A P S N L A
    TACCAGCAGA AGCCAGGATC CTCCCCAAA CCCTGGATTT ATGCCCCATC CAACCTGGCT

    S G V P A R F S G S G S G T S Y S L T I
241 TCTGGAGTCC CTGCTCGCTT CAGTGGCAGT GGGTCTGGGA CCTCTTACTC TCTCACAATC

    S R V E A E D A A T Y Y C Q Q W S F N P
301 AGCAGAGTGG AGGCTGAAGA TGCTGCCACT TATTACTGCC AGCAGTGGAG TTTTAACCCA

                        (Gly4Ser)3 Linker →
361  P T F G A G T K L E L K G G G G S G G G
    CCCACGTTTC GTGTGGGAC CAAGCTGGAG CTGAAAGGTG GCGGTGGCTC GGGCGGTGGT

                        2H7 VH →
421  G S G G G G S S Q A Y L Q Q S G A E L V
    GGATCTGGAG GAGGTGGGAG CTCTCAGGCT TATCTACAGC AGTCTGGGGC TGAGCTGGTG

    R P G A S V K M S C K A S G Y T F T S Y
481 AGGCCTGGGG CCTCAGTGAA GATGTCCTGC AAGGCTTCTG GCTACACATT TACCAGTTAC

    N M H W V K Q T P R Q G L E W I G A I Y
541 AATATGCACT GGGTAAAGCA GACACCTAGA CAGGGCCTGG AATGGATTGG AGCTATTTAT

    P G N G D T S Y N Q K F K G K A T L T V
601 CCAGGAAATG GTGATACTTC CTACAATCAG AAGTTCAAGG GCAAGGCCAC ACTGACTGTA

    D K S S S T A Y M Q L S S L T S E D S A
661 GACAAATCCT CCAGCACAGC CTACATGCAG CTCAGCAGCC TGACATCTGA AGACTCTGCG

    V Y F C A R V V Y Y S N S Y W Y F D V W
721 GTCTATTTCT GTGCAAGAGT GGTGTACTAT ACTAACTCTT ACTGGTACTT CGATGTCTGG
```


Figure 7B

human CD154/amino acid 48→

Bcl/Bam hybrid site

781 G T G T T V T V S D P R R L D K I E D E
GGCACAGGGA CCACGGTCAC CGTCTCTGAT CCAAGAAGGT TGGACAAGAT AGAAGATGAA

841 R N L H E D F V F M K T I Q R C N T G E
AGGAATCTTC ATGAAGATTT TGTATTTCATG AAAACGATAC AGAGATGCAA CACAGGAGAA

901 R S L S L L N C E E I K S Q F E G F V K
AGATCCTTAT CCTTACTGAA CTGTGAGGAG ATTAAAAGCC AGTTTGAAGG CTTTGTGAAG

BclI

961 D I M L N K E E T K K E N S F E M Q K G
GATATAATGT TAAACAAAGA GGAGACGAAG AAAGAAAACA GCTTTGAAAT GCAAAAAGGT

BclI
~~~~~

1021 D Q N P Q I A A H V I S E A S S K T T S  
GATCAGAATC CTCAAATTGC GGCACATGTC ATAAGTGAGG CCAGCAGTAA AACAACTCT

1081 V L Q W A E K G Y Y T M S N N L V T L E  
GTGTTACAGT GGGCTGAAAA AGGATACTAC ACCATGAGCA ACAACTTGGT AACCTTGAA

1141 N G K Q L T V K R Q G L Y Y I Y A Q V T  
AATGGGAAAC AGCTGACCGT TAAAGACAA GGACTCTATT ATATCTATGC CCAAGTCACC

HindIII  
~~~~~

1201 F C S N R E A S S Q A P F I A S L C L K
TTCTGTTCCA ATCGGGAAGC TTCGAGTCAA GCTCCATTTA TAGCCAGCCT CTGCCTAAAG

1261 S P G R F E R I L L R A A N T H S S A K
TCCCCGGTA GATTCGAGAG AATCTTACTC AGAGCTGCAA ATACCCACAG TTCCGCCAAA

1321 P C G Q Q S I H L G G V F E L Q P G A S
CCTTGCGGGC AACAAATCCAT TCACTTGGGA GGAGTATTG AATTGCAACC AGGTGCTTCG

NcoI
~~~~~

1381 V F V N V T D P S Q V S H G T G F T S F  
GTGTTTGTCA ATGTGACTGA TCCAAGCCAA GTGAGCCATG GCACTGGCTT CACGTCCTTT

XhoI                      XbaI  
~~~~~                      ~~~~~

1441 G L L K L E * * S R
GGCTTACTCA AACTCGAGTG ATAATCTAGA

Figure 7C

2H7scFv-CD154 S4 cDNA and predicted amino acid sequence:

```
HindIII      NcoI
~~~~~      ~~~~~2H7 VL Leader Peptide→
                M D F Q V Q I F S F L L I S A S
1  AAGCTTGCCG CC  ATGGATT TCAAGTGCAG ATTTTCAGCT TCCTGCTAAT CAGTGCTTCA

                2H7 VL →
        V I I A R G Q I V L S Q S P A I L S A S
61  GTCATAATTG CCAGAGGACA AATTGTTCTC TCCAGTCTC CAGCAATCCT GTCTGCATCT

        P G E K V T M T C R A S S S V S Y M H W
121 CCAGGGGAGA AGGTACAAT GACTGCAGG GCCAGTCAA GTGTAAGTTA CATGCACTGG

                BamHI
                ~~~~~
        Y Q Q K P G S S P K P W I Y A P S N L A
181 TACCAGCAGA AGCCAGGATC CTCCCCAAA CCCTGGATTT ATGCCCCATC CAACCTGGCT

        S G V P A R F S G S G S G T S Y S L T I
241 TCTGGAGTCC CTGCTCGCTT CAGTGGCAGT GGTCTGGGA CCTCTTACTC TCTACAATC

        S R V E A E D A A T Y Y C Q Q W S F N P
301 AGCAGAGTGG AGGCTGAAGA TGCTGCCACT TATTACTGCC AGCAGTGGAG TTTTAACCCA

                (Gly4Ser)3 Linker →
        P T F G A G T K L E L K G G G G S G G G
361 CCCACGTTG GTGCTGGGAC CAAGCTGGAG CTGAAAGGTG GCGGTGGCTC GGGCGGTGGT

                2H7 VH →
        G S G G G G S S Q A Y L Q Q S G A E L V
421 GGATCTGGAG CAGGTGGGAG CTCTCAGGCT TATCTACAGC AGTCTGGGGC TGAGCTGGTG

        R P G A S V K M S C K A S G Y T F T S Y
481 AGGCCTGGGG CCTCAGTGAA GATGTCCTGC AAGGCTTCTG GCTACACATT TACCAGTTAC

        N M H W V K Q T P R Q G L E W I G A I Y
541 AATATGCACT GGGTAAAGCA GACACCTAGA CAGGCCTGG AATGGATTGG AGCTATTTAT

        P G N G D T S Y N Q K F K G K A T L T V
601 CCAGGAAATG GTGATACTTC CTACAATCAG AAGTTCAAGG GCAAGGCCAC ACTGACTGTA

        D K S S S T A Y M Q L S S L T S E D S A
661 GACAAATCCT CCAGCACAGC CTACATGCAG CTCAGCAGCC TGACATCTGA AGACTCTGCG

        V Y F C A R V V Y Y S N S Y W Y F D V W
721 GTCTATTTCT GTGCAAGAGT GGTGTACTAT AGTAACTCTT ACTGGTACTT CGATGTCTGG
```

Figure 7D.

human CD154/amino acid 108 →

```

                                Bcl/Bam hybrid site                                BclI
      G T G T   T V T   V S D   P E N S   F E M   Q K G
781  GGCACAGGGA CCACGGTCAC CGTCTCTGAT CCAGAAAACA GCTTTGAAAT GCAAAAAGGT

      BclI
      ~~~~~
      D Q N P   Q I A   A H V   I S E A   S S K   T T S
841  GATCAGAATC CTCAAATTGC GGCACATGTC ATAAGTGAGG CCAGCAGTAA AACACATCT

      V L Q W   A E K   G Y Y   T M S N   N L V   T L E
901  GTGTTACAGT GGGCTGAAAA AGGATACTAC ACCATGAGCA ACAACTTGGT AACCTGGAA

      N G K Q   L T V   K R Q   G L Y Y   I Y A   Q V T
961  AATGGGAAAC AGCTGACCGT TAAAAGACAA GGACTCTATT ATATCTATGC CCAAGTCACC

                                HindIII
                                ~~~~~
      F C S N   R E A   S S Q   A P F I   A S L   C L K
1021 TTCTGTTCCA ATCGGGAAGC TTCGAGTCAA GCTCCATTTA TAGCCAGCCT CTGCCTAAAG

      S P G R   F E R   I L L   R A A N   T H S   S A K
1081 TCCCCCGGTA GATTCGAGAG AATCTTACTC AGAGCTGCAA ATACCCACAG TTCCGCCAAA

      P C G Q   Q S I   H L G   G V F E   L Q P   G A S
1141 CCTTGCGGGC AACAATCCAT TCACTTGGGA GGAGTATTTG AATTGCAACC AGGTGCTTCG

                                NcoI
                                ~~~~~
      V F V N   V T D   P S Q   V S H G   T G F   T S F
1201 GTGTTTGTCA ATGTGACTGA TCCAAGCCAA GTGAGCCATG GCACTGGCTT CACGTCCTTT

                                XhoI                                XbaI
                                ~~~~~                                ~~~~~
      G L L K   L E *   * S R
1261 GGCTTACTCA AACTCGAGTG ATAATCTAGA
```

Figure 8.

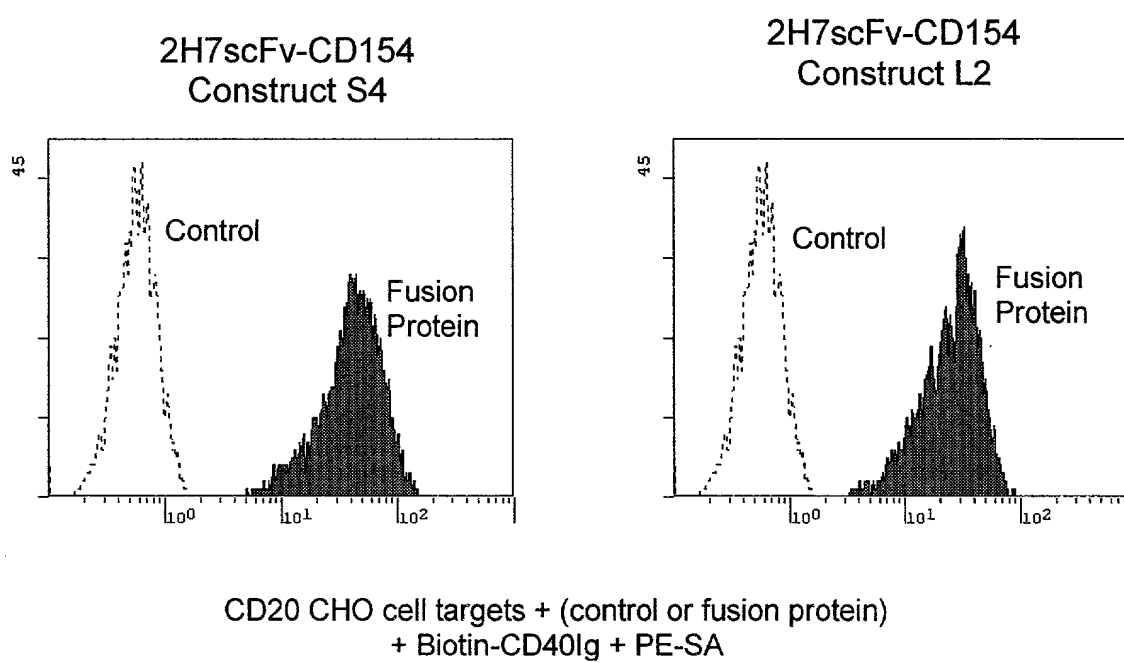
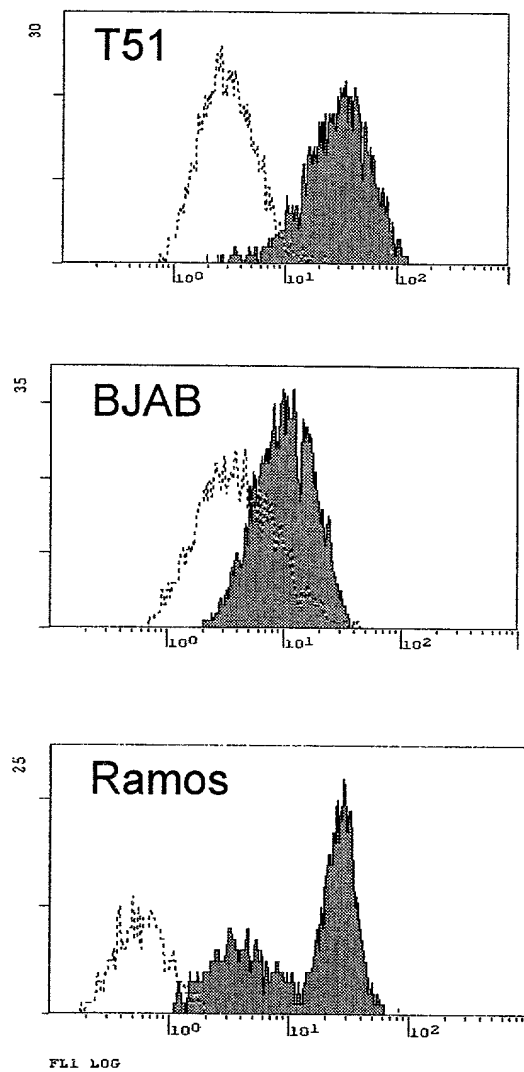


Figure 9.



.....control supernatant ____2H7scFv-CD154 supernatant

Figure 10.

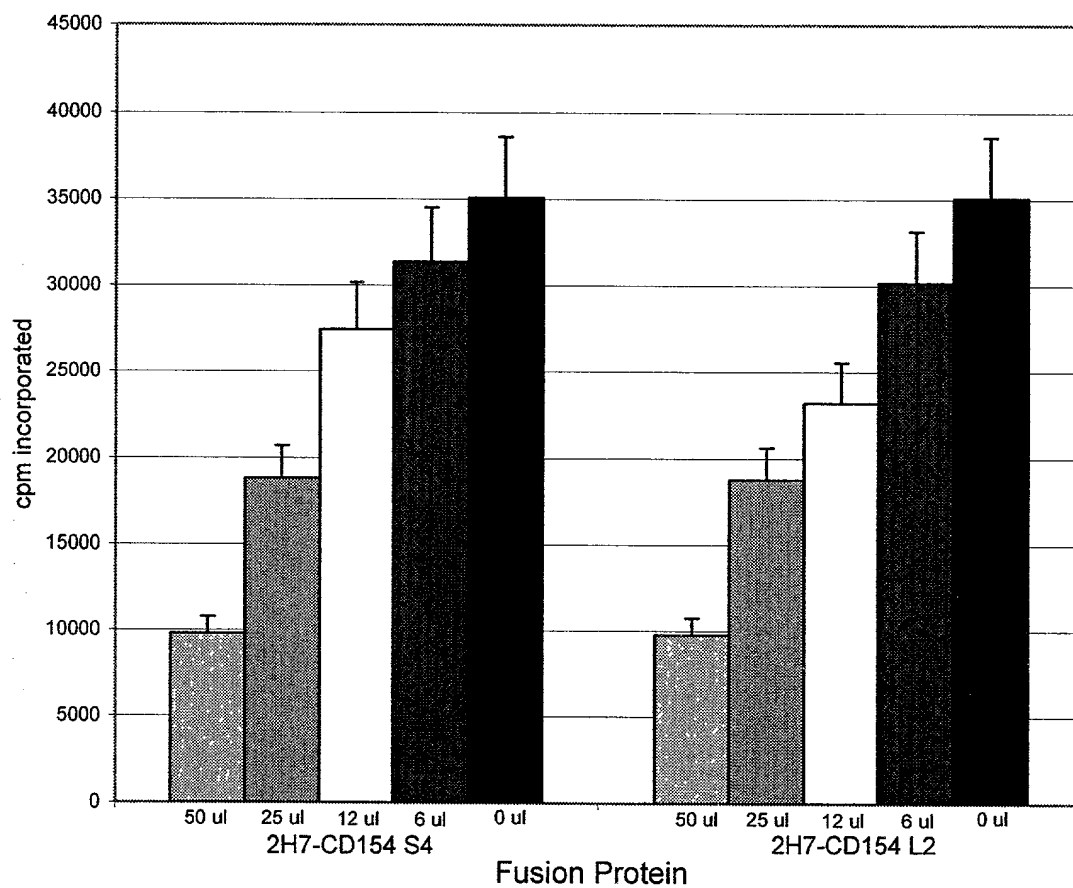
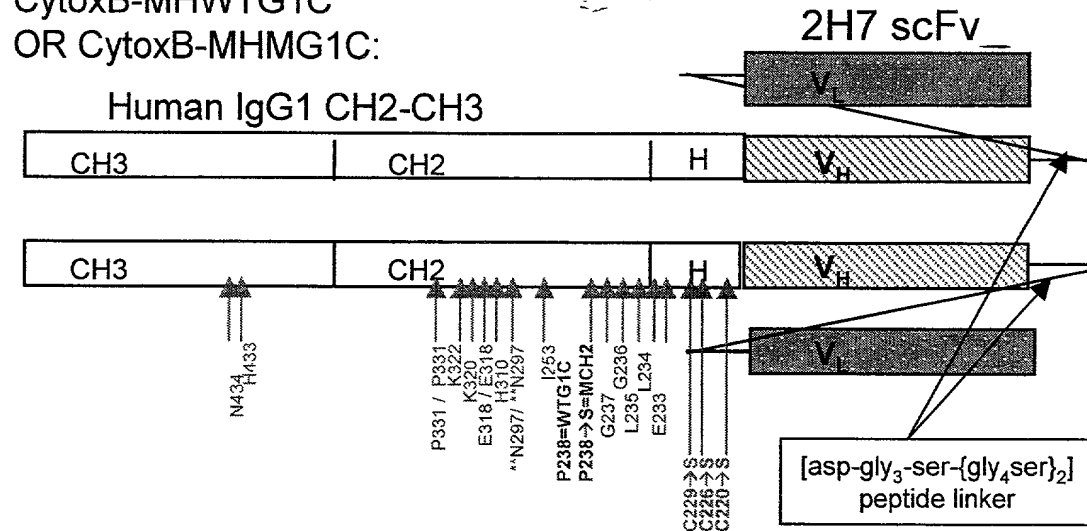
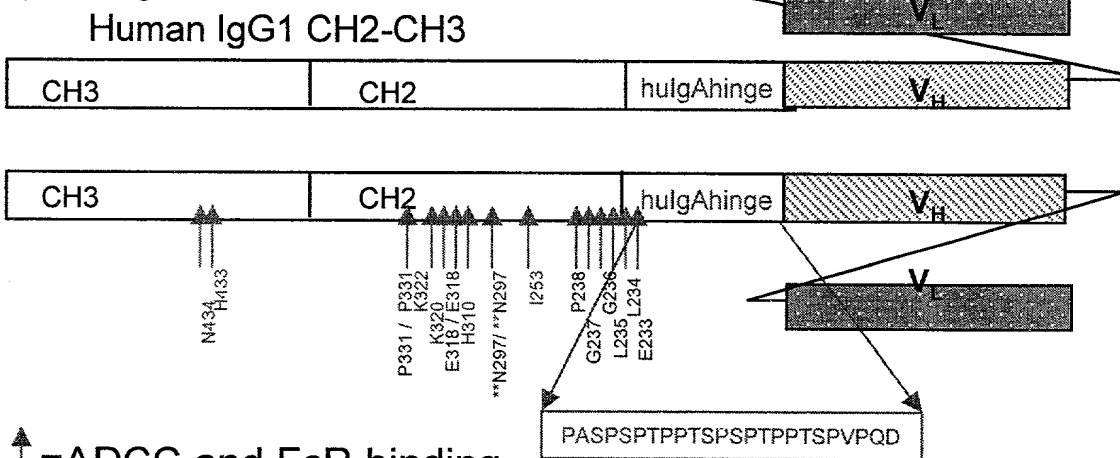


FIGURE 11

CytoxB-MHWTG1C
 OR Cytox-B-MHMG1C:



CytoxB-IgAHWTHG1C:



↑ =ADCC and FcR binding

↑ =Complement Fixation

FIGURE 12

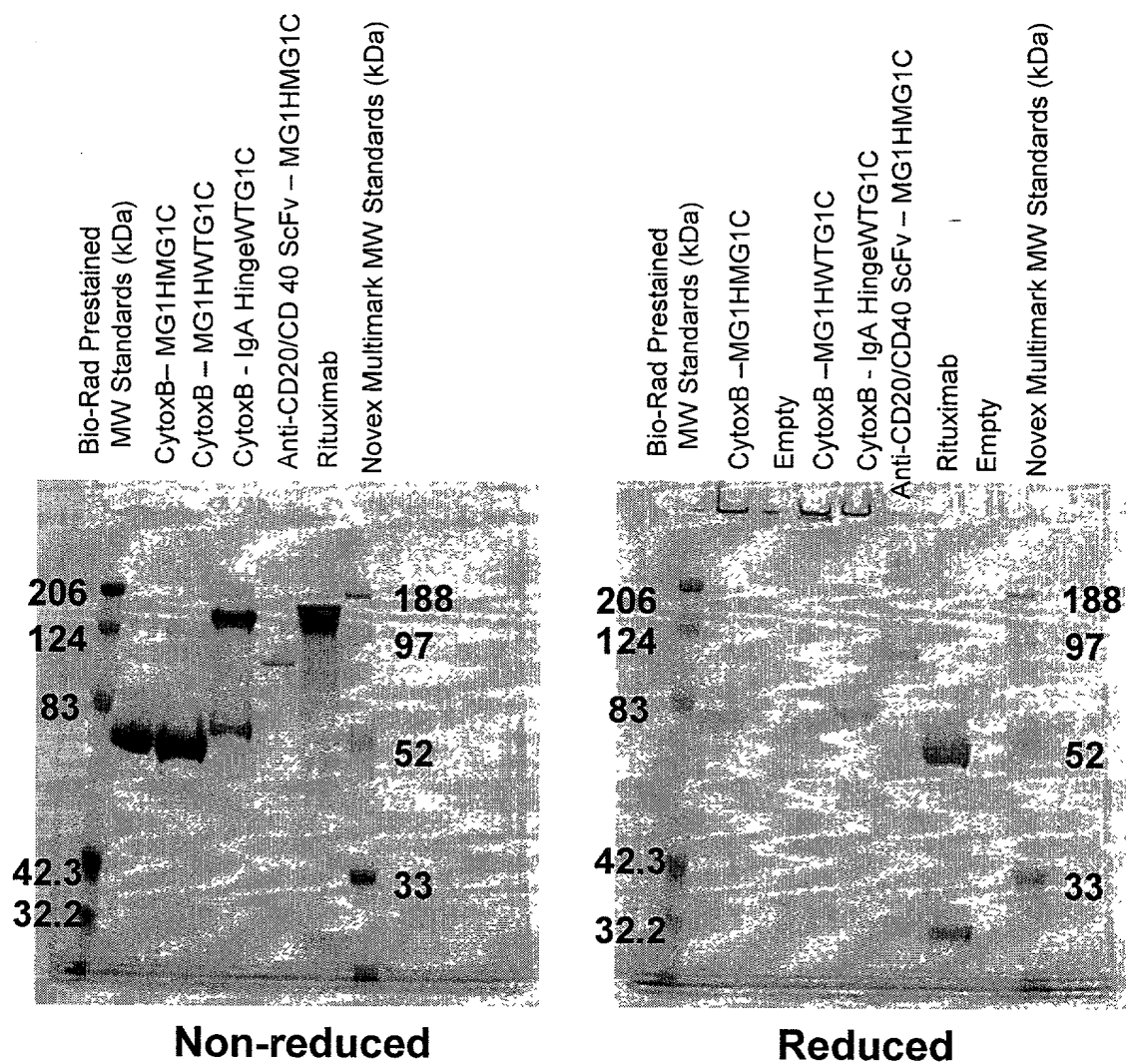


FIGURE 13

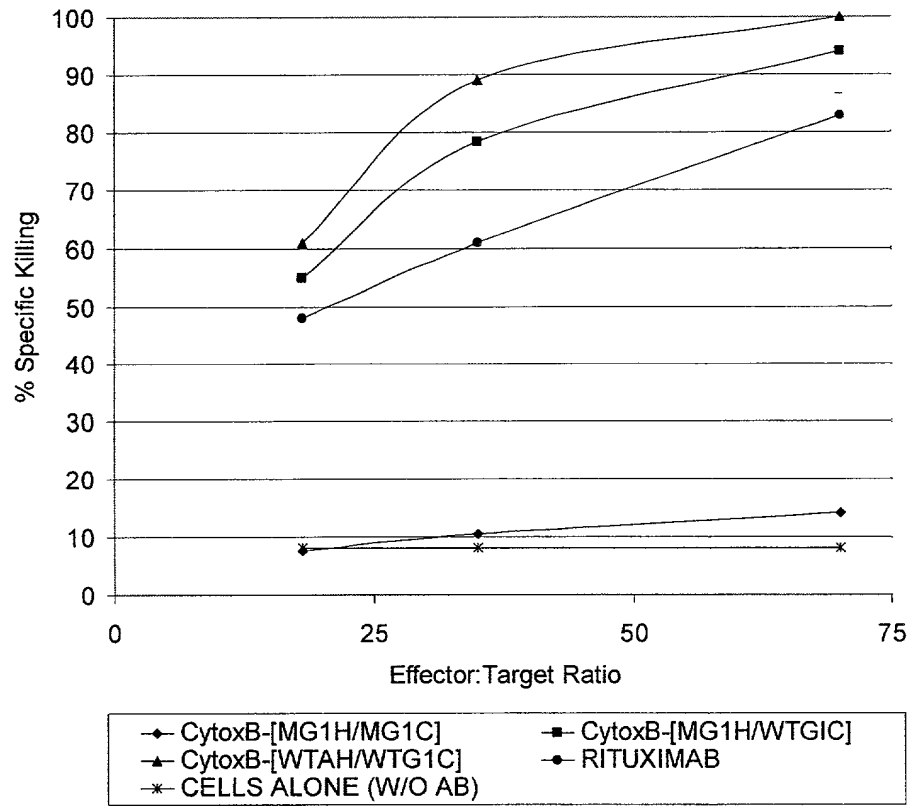
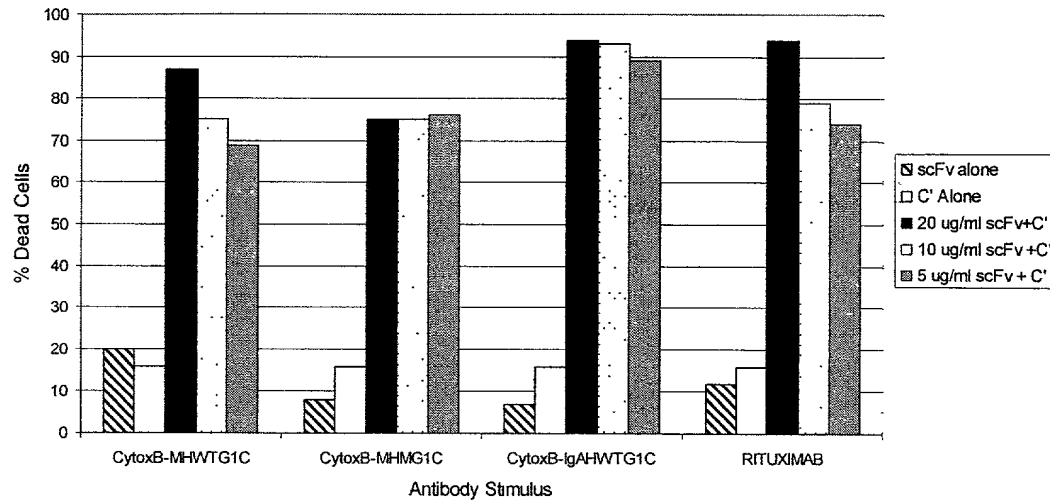
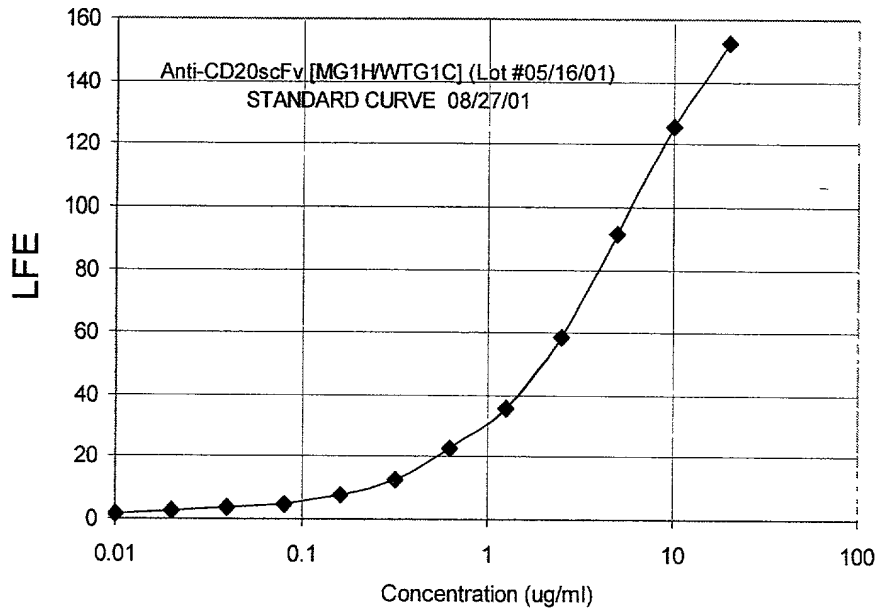


FIGURE 14





| | | Monkey J99231 | | Monkey K99334 | |
|-------------|----|---------------|---------------------------------------|---------------|---------------------------------------|
| Day | | LFE(1:40) | Concentration
($\mu\text{g/mL}$) | LFE(1:40) | Concentration
($\mu\text{g/mL}$) |
| Injection → | -7 | 2.41 | <0.6 $\mu\text{g/mL}$ | 1.51 | <0.4 $\mu\text{g/mL}$ |
| | 0 | 2.22 | <0.6 $\mu\text{g/mL}$ | 1.63 | <0.4 $\mu\text{g/mL}$ |
| Injection → | 1 | 73.8 | 220 $\mu\text{g/mL}$ | 44.4 | 100 $\mu\text{g/mL}$ |
| | 3 | 20.0 | 28 $\mu\text{g/mL}$ | 40.2 | 80 $\mu\text{g/mL}$ |
| | 7 | 15.6 | 24 $\mu\text{g/mL}$ | 15.7 | 24 $\mu\text{g/mL}$ |
| | 8 | 39.1 | 80 $\mu\text{g/mL}$ | 42.6 | 92 $\mu\text{g/mL}$ |
| | 10 | 11.5 | 18 $\mu\text{g/mL}$ | 2.74 | 1.2 $\mu\text{g/mL}$ |
| | 14 | 2.05 | 0.6mg/mL | 1.96 | 0.6 $\mu\text{g/mL}$ |

Figure 16

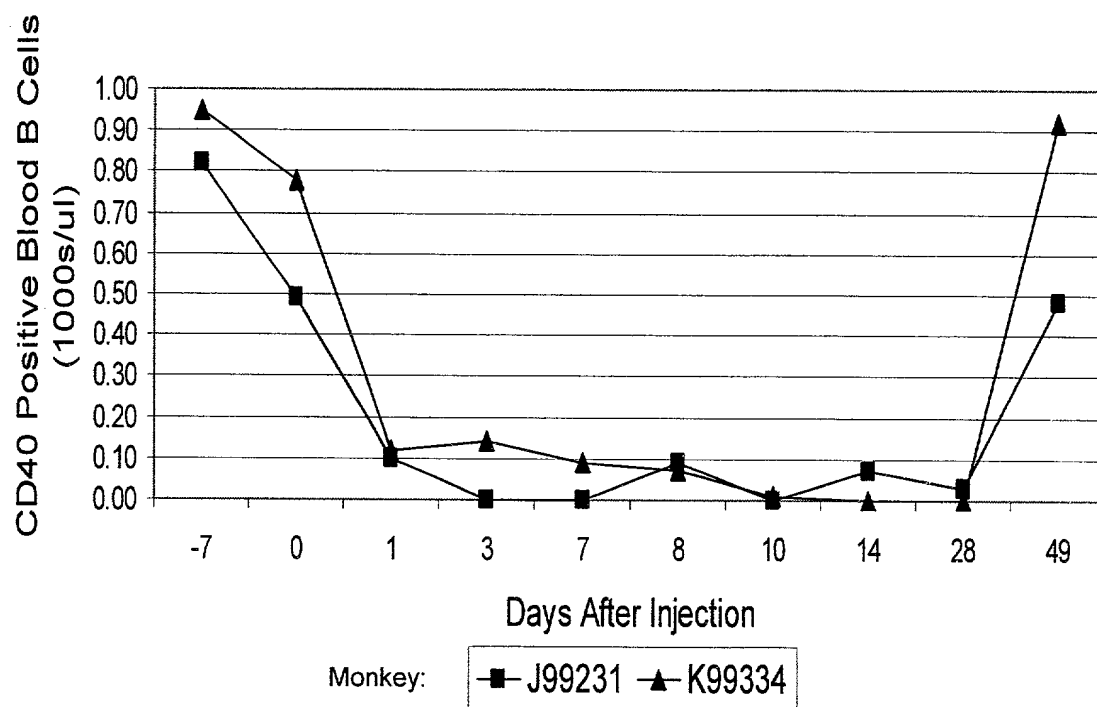
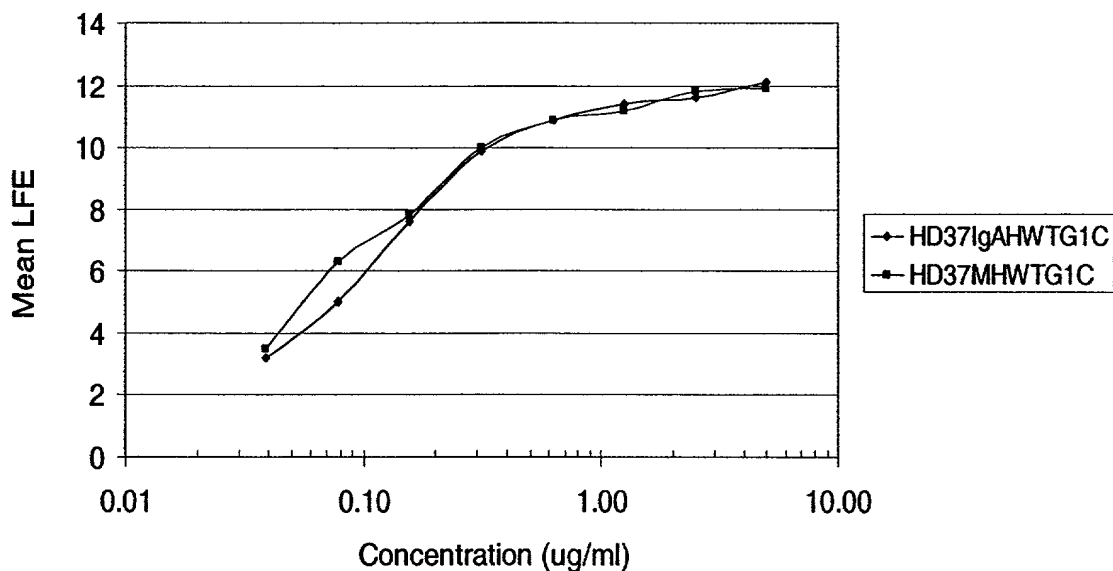


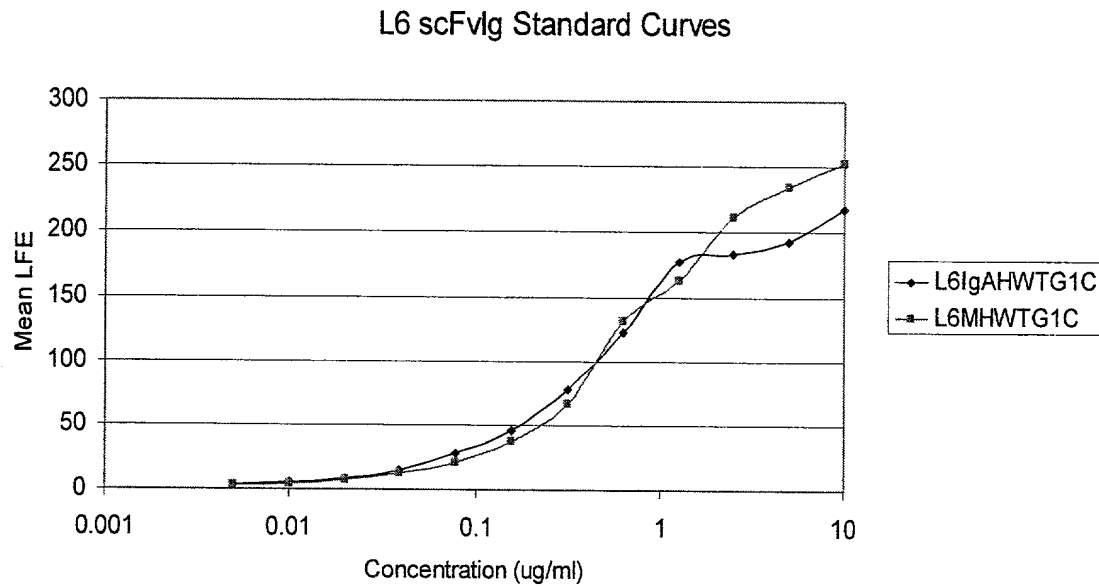
FIGURE 17

Standard Curve of HD37 scFvlg Derivative
 Binding to B Cells



| Clone/Isolate | Mean LFE at 1:100 | Estimated Concentration |
|----------------|-------------------|-------------------------|
| Bulk IgAHTWG1C | 11.2 | > 60 ug/ml |
| 1B2 | 10.4 | >50 ug/ml |
| 6C5 | 10.5 | >50 ug/ml |
| 4B1 | 8.6 | >40 ug/ml |
| Bulk MHTWG1C | 10.9 | > 50 ug/ml |
| 2G8 | 10.6 | > 50 ug/ml |
| 3F3 | 8.3 | >40 ug/ml |
| 3D9 | 11.1 | > 60 ug/ml |

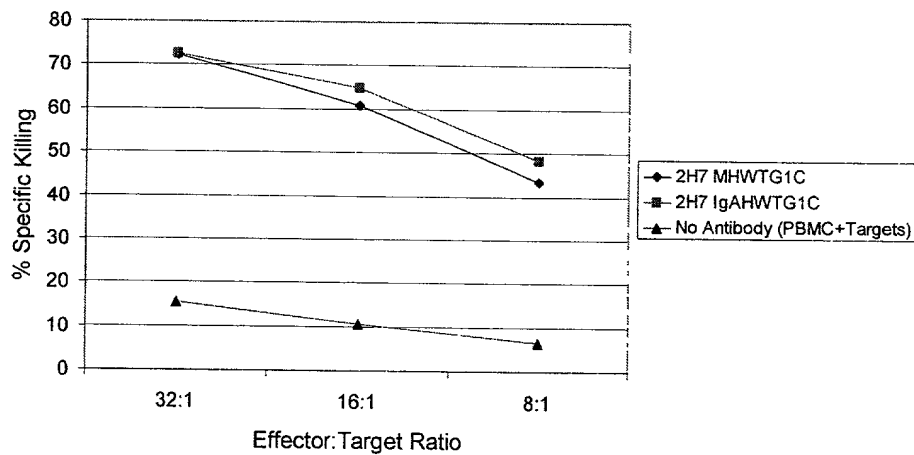
FIGURE 18



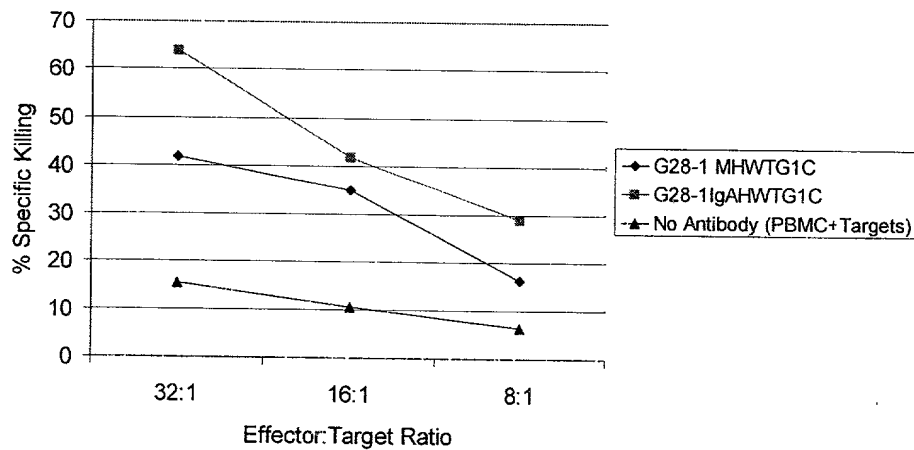
| Construct | Mean LFE 1:20 | Estimated Concentration |
|-------------------------------------|---------------|-------------------------|
| L6IgAHWTG1C
unamplified CHO sup | 51.1 | 6.25 ug/ml |
| L6IgGMHWTG1C
unamplified CHO sup | 23.0 | 3.2 ug/ml |

FIGURE 19

A. 2H7 (anti-CD20) scFv Derivatives



B. G28-1 (anti-CD37) scFv Derivatives



C. HD37 (anti-CD19) scFv Derivatives

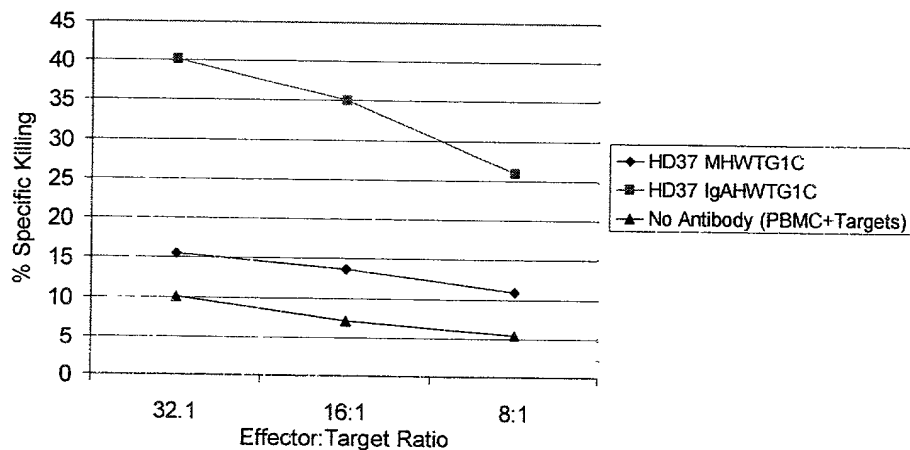


FIGURE 20

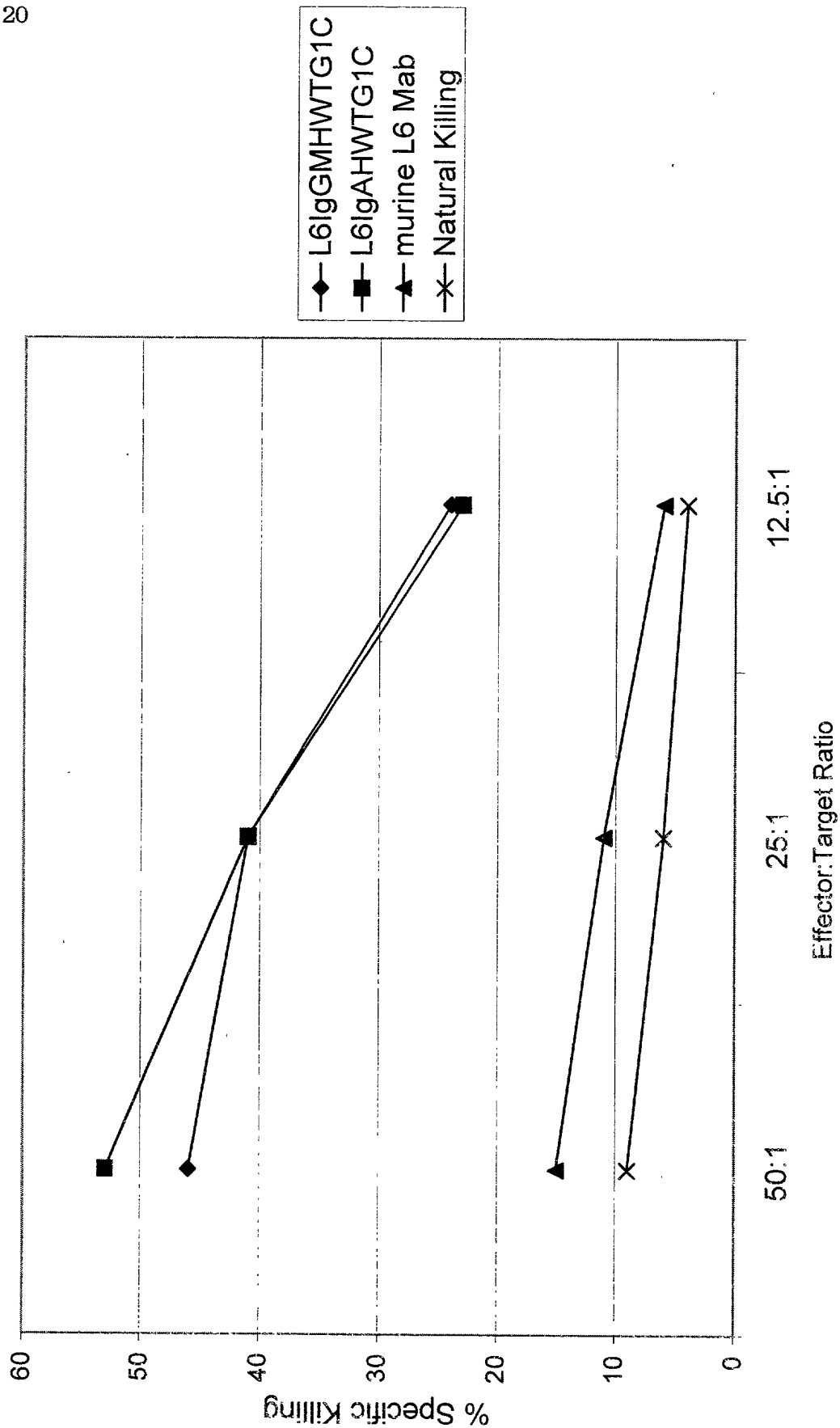


Figure 21

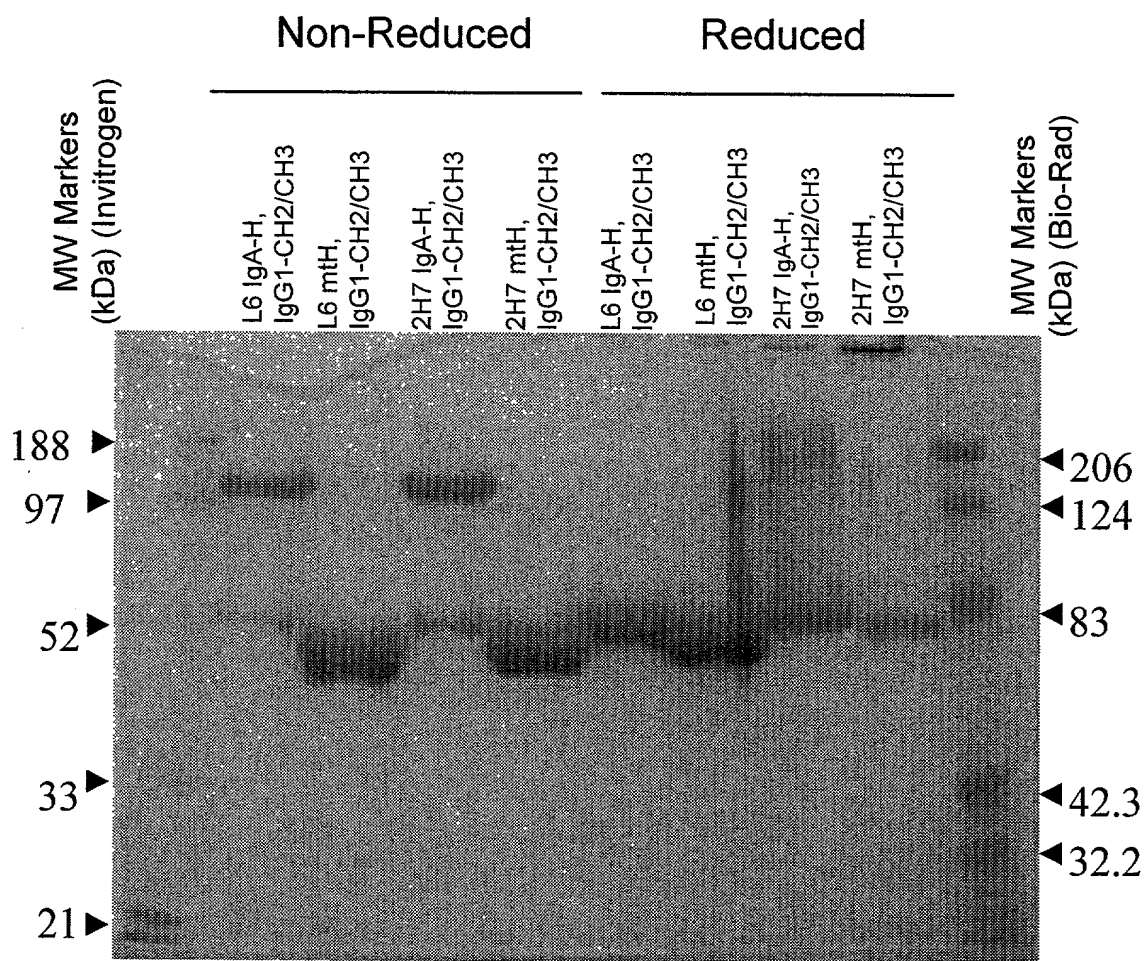


Figure 22

